Vol. LXXIX, No. 2 October, 1940

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

DECOMPOSITION AND REGENERATION OF NITROGENOUS ORGANIC MATTER IN SEA WATER[1]

III. Influence of Temperature and Source and Condition of Water

THEODOR VON BRAND AND NORRIS W. RAKESTRAW

(*From the Woods Hole Oceanographic Institution, Woods Hole, Mass.*)

The first two papers in this series (1937, 1939) have shown that the decomposition of suspended organic matter is accompanied by the successive formation of ammonia, nitrite and nitrate in the sea water medium in distinctly recognizable stages; that fresh diatoms can grow in the water at any stage in the cycle; and that successive cycles of decomposition and regeneration can be carried out in the same water, without the addition of new organic substrate. Since the analogous processes in the sea can scarcely be expected to take place in such simple, successive fashion, it is important to know how the decomposition cycle is influenced in rate or character by a number of variable factors. Among these are temperature, source of the sea water, and the presence or absence of such micro-organisms as can be controlled by the routine technic of sterilization. The present paper describes an experimental study of these factors.

Earlier work had shown that the nature of the decomposition cycle varied somewhat with the source of the water, being generally more rapid in water from the harbor than in that from offshore. To pursue this point further three samples of water were taken from a deep-sea station (25°–32′ N.; 53°–45′ W.): one from the surface, one from the level of the oxygen minimum (800 meters), and one from 1200 meters. Each of these samples was divided into two parts, one of which was "sterilized" by suction filtration through a No. 5 sintered-glass funnel. There were previous indications that this treatment was adequate to remove bacteria.

To each of the six portions of sea water (about two liters each) were added equal amounts of washed *Nitzschia Closterium,* which, as in

[1] Contribution No. 268 from the Woods Hole Oceanographic Institution.

previous experiments, served as a convenient source of organic matter. The suspensions were placed in the dark, at laboratory temperature, and periodically analyzed for particulate nitrogen, ammonia, nitrite and nitrate, in the manner already described. The results are shown in Fig. 1.

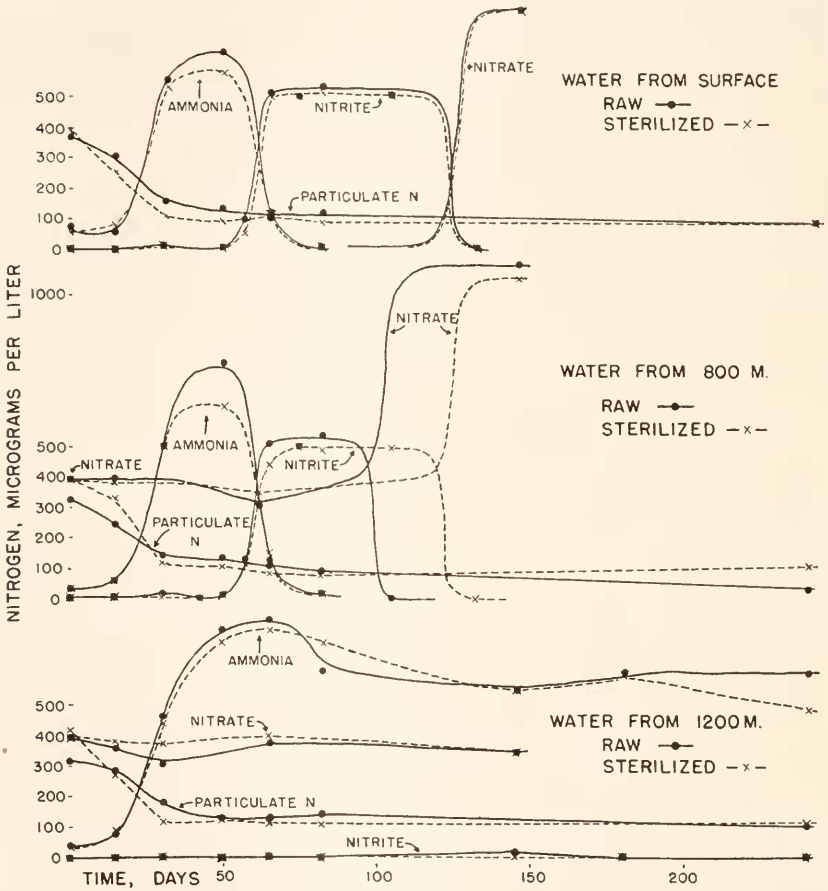

FIG. 1. The disappearance of particulate nitrogen and the simultaneous changes in ammonia, nitrite and nitrate. Fresh cultures of *Nitzschia Closterium* suspended in sea water from different depths, part of which has been sterilized by filtration.

There was no difference in the behavior of those samples which had been sterilized by filtration and those which had not. The decomposition proceeded in the normal manner in the surface water and in that from the oxygen minimum layer. But in the deep water (1200 m.)

there was no oxidation of ammonia during the time of the experiment (250 days). The nature of this inhibiting effect is yet to be determined. That it is not due simply to the absence of bacteria seems evident from the fact that such oxidation did proceed in the other samples, whether or not the water had been sterilized. This would indicate that the necessary organisms were carried by the diatoms. But since the same diatom culture was used in the deep water, the failure of oxidation to take place is evidently due to some other, unexplained factor. It should be pointed out that ammonia and nitrite are seldom found below 200 or 300 meters in the sea.

Since the oxidizing organisms seemed to be carried by the diatoms rather than in the water itself, another series of experiments was carried out in which harbor water was used as the suspension medium, part of which was sterilized by filtration, as before. To portions of the sterile and the raw water washed diatoms were added, as in the last series. To other similar portions of the water (sterile and raw) were added equal amounts of a diatom suspension which had been boiled for two minutes to sterilize it. Precautions were taken to insure the addition of equal amounts of organic matter to all four of the cultures, after which they were placed in the dark and sampled periodically, according to the usual routine. The results are shown in Fig. 2.

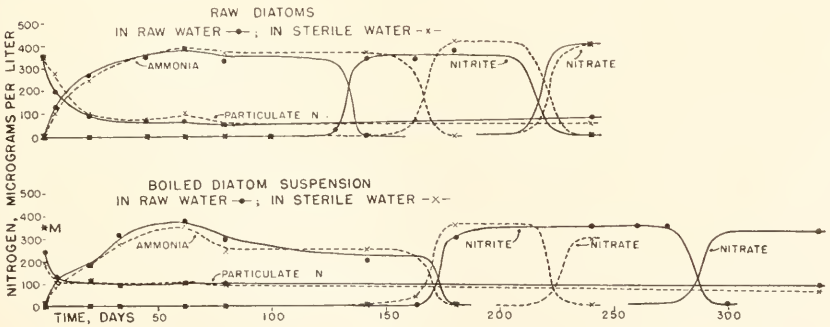

Fig. 2. The disappearance of particulate nitrogen and the simultaneous changes in ammonia, nitrite and nitrate, in harbor water, one portion sterilized by filtration. Upper curves: fresh cultures of *Nitzschia Closterium*. Lower curves: *Nitzschia* cultures boiled to sterilize. The point marked *M indicates the amount of particulate nitrogen in the *Nitzschia* culture before boiling.

The boiling of the diatom suspension quite evidently throws some of the organic matter into solution, for, although equal quantities of diatoms were used, the first determination of particulate nitrogen is much lower in the cultures to which the boiled suspensions were added than in those containing the same amount of raw diatom material. The

boiling of the diatoms also resulted in a somewhat slower rate of ammonia formation and a prolongation of the ammonia stage of the cycle.

The fact that the decomposition cycles were so nearly alike in the sterilized and the raw sea water, and that oxidation of ammonia to nitrite and nitrate took place in all cases, raises the serious question whether the filtration had actually sterilized the sea water, or whether the cultures had been contaminated with nitrite-forming organisms from the air. It is difficult to explain the formation of nitrite and nitrate except upon one of these two assumptions and we are not as yet able to resolve this difficulty. The culture methods for the identification of nitrite-forming bacteria are so unsatisfactory that we did not attempt to use them upon our material. It is almost impossible to use aseptic technic in sampling the cultures, although the chance of contamination can be diminished by pouring rather than pipetting samples.

In the effort to get further information on this point, four supposedly bacteria-free cultures of *Nitzschia* were obtained. One of these was analyzed immediately and found to be free of ammonia, nitrite and nitrate. The others were placed in the dark and analyzed later. After 145 days one culture contained 500 γ of ammonia nitrogen and 10 γ of nitrite nitrogen per liter. After 300 days the second contained 870 γ of ammonia nitrogen and 35 γ of nitrite. A large amount of suspended material still remained in both cases, but it was evident that considerable decomposition had taken place.

The last culture was opened and large numbers of bacteria were found in the remaining suspended matter. Even with great care we had not succeeded in preparing and preserving sterile cultures. Nevertheless, it seems worthwhile to note that bacteria developed only very slowly and that an almost negligible amount of ammonia had been oxidized to nitrite in 300 days. Apparently it is easier to eliminate the organisms responsible for oxidation than those which may possibly take part in the formation of ammonia.

To determine the temperature coefficient of the decomposition process, three suspensions of washed cells of *Nitzschia* were prepared in the usual manner. These were then placed in the dark at constant temperatures: one at 15° C., one at 7–9° C., and one at 1–2° C. By a stroke of good luck, when the first complete analysis was made, after 280 days, the one at the lowest temperature (No. 28) was in the ammonia stage, the one at the middle temperature (No. 29) was in the nitrite stage, and the one at the highest temperature (No. 30) had completed the cycle and all the nitrogen was in the form of nitrate. The complete results are given in Table I.

Unfortunately, No. 29 was lost at about the time when the nitrite had disappeared, but the cycle was so nearly completed at the time of the last preceding analysis that a sufficiently accurate estimate of the total elapsed time could be made. At the date of writing, after 635 days, No. 28 had not gone beyond the ammonia stage.

TABLE I

Series Nos. 28, 29 and 30. Sea water containing fresh cultures of *Nitzschia Closterium* kept in the dark at different temperatures as shown. Micrograms of nitrogen per liter.

	Date	Particulate	Ammonia	Nitrite	Nitrate
No. 28	9–14–38	470	10	0	10
Temperature	10– 3–38	260			
1 to 2° C.	1– 6–39	244			
	4–17–39	98			
	6–22–39	134	385	0	15
	8–30–39	64			
	2–26–40	24	310	0	10
	6–11–40	24	385	0	5
No. 29	9–14–38	470	10	0	10
Temperature	10– 3–38	129			
7 to 9° C.	1– 6–39	75			
	4–17–39	51			
	6–22–39	67	21	330	10
	8–30–39	41	20	320	
	9– 7–39			200	
	2–26–40	25	0	45	
No. 30	9–14–38	483	5	0	10
Temperature	10– 3–38	38			
15° C.	1– 6–39	49			
	4– 7–39	47			
	6–22–39	57	18	0	375
	8–30–39	28	25	0	400
	2–26–40	40	0	0	400
	6–11–40	18	0	0	400

The complete cycle in No. 30 (15°) took less than 280 days, and in No. 29 (7–9°) about 550 days. A temperature difference of 6 to 8° C. therefore more than doubled the velocity of the over-all process. Since more than one reaction mechanism is involved in the cycle, the relation is probably not a simple one, and it is indeed possible that a certain minimum temperature is necessary for the oxidation of ammonia.

On September 2, 1939, portions of cultures Nos. 28, 29 and 30 were inoculated with fresh diatoms and placed in the light. At this time the maximum amounts of ammonia, nitrite and nitrate, respectively, were

in these three cultures. Two weeks later there was no ammonia, nitrite or nitrate in any of the three, and an abundant diatom growth was observed. This confirms definitely our earlier observation that new diatoms can grow in the medium at any stage in the cycle of decomposition.

Summary

The source of the water is important in determining the nature of the decomposition cycle. Oxidation of ammonia to nitrite is retarded in water from the deep sea (1200 m.). Inconclusive results were obtained from efforts to sterilize both the water and the original organic matter, but it is evidently easier to eliminate organisms responsible for the oxidation processes than those which may participate in the formation of ammonia. The speed of the whole decomposition cycle was more than doubled by an increase of 6° or 8° in temperature. Growth of diatoms is possible at any stage in the cycle of decomposition.

BIBLIOGRAPHY

VON BRAND, T., N. W. RAKESTRAW AND C. E. RENN, 1937. The experimental decomposition and regeneration of nitrogenous organic matter in sea water. *Biol. Bull.*, **72**: 165–175.

VON BRAND, T., N. W. RAKESTRAW AND C. E. RENN, 1939. Further experiments on the decomposition and regeneration of nitrogenous organic matter in sea water. *Biol. Bull.*, **77**: 285–296.